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Morphometric Study of a Hybrid Population of *Acacia verniciflua* and *A. aspera* (*Acacia* subgenus *Phyllodineae*)

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Abstract

Hybrids between the common variant of *Acacia verniciflua* A.Cunn. and *Acacia aspera* Lindl. are reported from central Victoria, in remnant vegetation in the median strip of the Hume Highway, south of Benalla. Plants of both species and suspected hybrids were sampled and mapped along a belt transect. Morphological pattern analysis, based largely on phyllode characters and using ordination and agglomerative classification techniques, confirmed a hybrid population of plants intermediate between *A. verniciflua* and *A. aspera* and possible backcrosses to one or other of the parent species. Scanning electron microscopy revealed that hybrids had density of simple hair and morphology of glandular trichomes intermediate between the two parents. The site has been disturbed, which may account for the hybrid swarm.

Introduction

Acacia verniciflua A.Cunn. (Varnish Wattle) occurs in South Australia, Queensland, New South Wales, Victoria, and Tasmania Fig. 1 (Fig. 1; Maslin 2001). It is a viscid shrub to small tree (1-8 m high) and highly variable across its range, with four variants in Victoria: common, southern, Casterton and Bacchus Marsh (Entwisle *et al.* 1996). Phyllodes are characterised by two main veins and glands (presumably resin-secreting), which are stalkless or sunken.

Acacia aspera Lindl. (Rough Wattle) is a spreading viscid shrub (0.5-2 m high) found in New South Wales and Victoria. It is less variable than *A. verniciflua* (Maslin 2001) with a single, newly recognised subspecies, *Acacia aspera* subsp. *parviceps*, found in

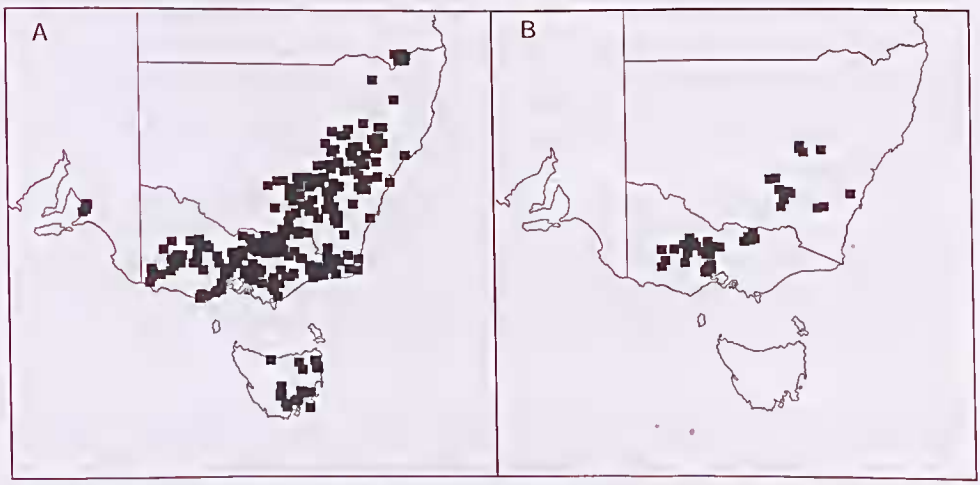

Figure 1. Geographic distribution of *Acacia verniciflua* and *A. aspera* in south-eastern mainland Australia.

Figure 2. Location of the study site, the Hume Highway, south of Benalla, Victoria.

Victoria in the Brisbane Ranges and near Beaufort (Walsh, 2004). Phyllodes have one main vein and flower buds have acuminate exserted bracteoles (Entwisle *et al.* 1996). The species is characterised by numerous simple hairs and glandular trichomes covering vegetative and reproductive structures. The latter are variously described as glandular hairs (Boughton 1989) or coarse hairs, gland-tipped (Entwisle *et al.* 1996).

Both *A. verniciflua* and *A. aspera* are classified in section *Phyllodineae* and are considered related (Maslin 2001). Entwisle *et al.* (1996) placed *A. verniciflua* in Group 3 and *A. aspera* in Group 4 of their treatment, although these groups are informal and do not necessarily reflect natural relationships. The geographic ranges of the two species overlap and in some localities they are sympatric. A mixed-species population of the common variant of *A. verniciflua*, *A. aspera*, and plants intermediate in morphology, has been found in central Victoria, south of Benalla (Fig. 2). The vegetation is remnant eucalypt woodland in the median strip of the Hume Highway and the site appears to have been disturbed (e.g. soil and drainage) by road-making. Given that *A. verniciflua* and *A. aspera* flower at the same time of the year, between July and November (Entwisle *et al* 1996), it was hypothesised that the intermediate plants are of hybrid origin between these two putative parents.

Natural hybridisation in *Acacia* Miller has been reported by a number of authors (Cheel 1935; Moffett 1956; Leach & Whiffin 1978; Ali & Qaiser 1980; Sedgley *et al.* 1992, Entwisle *et al.* 1996). Hybrids between *A. aspera* and *A. montana* have been recorded from the Whipstick, near Bendigo, Victoria (Maslin 2001), but no record of hybridisation between *A. verniciflua* and *A. aspera* has been reported to date.

Method

The study site is located 185km north of Melbourne along the Hume Highway, 3 km north east of Erreys road, on the median strip between the north- and south-bound lanes (Latitude: S 36° 36' 11", Longitude: E 145° 49' 30"). The remnant vegetation is dominated by *Eucalyptus microcarpa* (Maiden) Maiden, *E. melliodora* A.Cunn. ex

Schauer, *E. polyanthemos* Schauer, *E. blakelyi* Maiden and *E. macrorhyncha* F.Muell. ex Benth. *Acacia* species at the site include *A. verniciflua*, *A. aspera*, *A. pycnantha* Benth. and *A. genistifolia* Link, and grasses are predominantly *Austrodanthonia* spp.

A 40 m belt transect line running north-south, parallel with the road, was used to sample the *A. aspera* and *A. verniciflua* population in the median strip. Sampling was done in April 2003, and no flowers or legumes were present. Some plants showed signs of severe drought stress. Every healthy plant within 2m to the east of the transect line was sampled. The height of the plant and the distance along the transect line where it was found were recorded. A sample of branchlets with phyllodes was taken from each plant for morphological measurements. Plants were sampled for voucher herbarium specimens (RP1-35 lodged at MELU). One plant typical of each of *A. aspera* and *A. verniciflua* and four suspected hybrids were also sampled for phyllode preservation in 70% ethanol.

Thirty-five specimens collected from the study site were measured for 12 phyllode, branchlet and bud characters (Table 1). Forty additional specimens from the National Herbarium of Victoria were also measured (Appendix 1, numbered 36 - 75), consisting of twenty specimens each of *Acacia verniciflua* and *A. aspera*, and including 10 samples from the north-east of Victoria. Five phyllodes were measured from each specimen, while bud and branchlet characters were scored by scanning the entire specimen. Averages of the measurements were taken in order to produce an individual score for each specimen.

Table 1. Characters measured for morphological analysis.

1.	Phyllode length (mm)
2.	Phyllode width at widest point (mm)
3.	Phyllode curvature (falcate or not): widest distance of inner edge of phyllode from a vertical line between base and tip of phyllode (mm)
4.	Distance from base of phyllode to the widest point of phyllode (mm)
5.	Distance from base of phyllode to the marginal gland (mm)
6.	Phyllode thickness (mm)
7.	Number of main veins on phyllode
8.	Length of pulvinus (mm)
9.	Resin glands stalked or sessile
10.	Branchlets distinctly tomentose (hairs) or not
11.	Flower buds with braeteoles exserted or not
12.	Pedunele and buds hairy or not

The computer package PATN (Belbin 1995) was used for phenetic pattern analysis of the data. The Manhattan metric distance measure was used to construct a distance matrix and both unweighted pair group method of averaging (UPGMA) and non-metric multidimensional scaling (NMDS) were used for classification and ordination analyses (Clifford and Stephenson 1975).

Samples of the phyllodes preserved in 70% ethanol were prepared for Scanning Electron Microscopy (SEM). Samples were gradually dried out through a series of 70%, 80%, 90% and 100% ethanol. Sections were critical point dried, mounted onto stubs with an adhesive carbon disc, sputter coated with a thin coating of gold and viewed under a X1-30 Philips SEM.

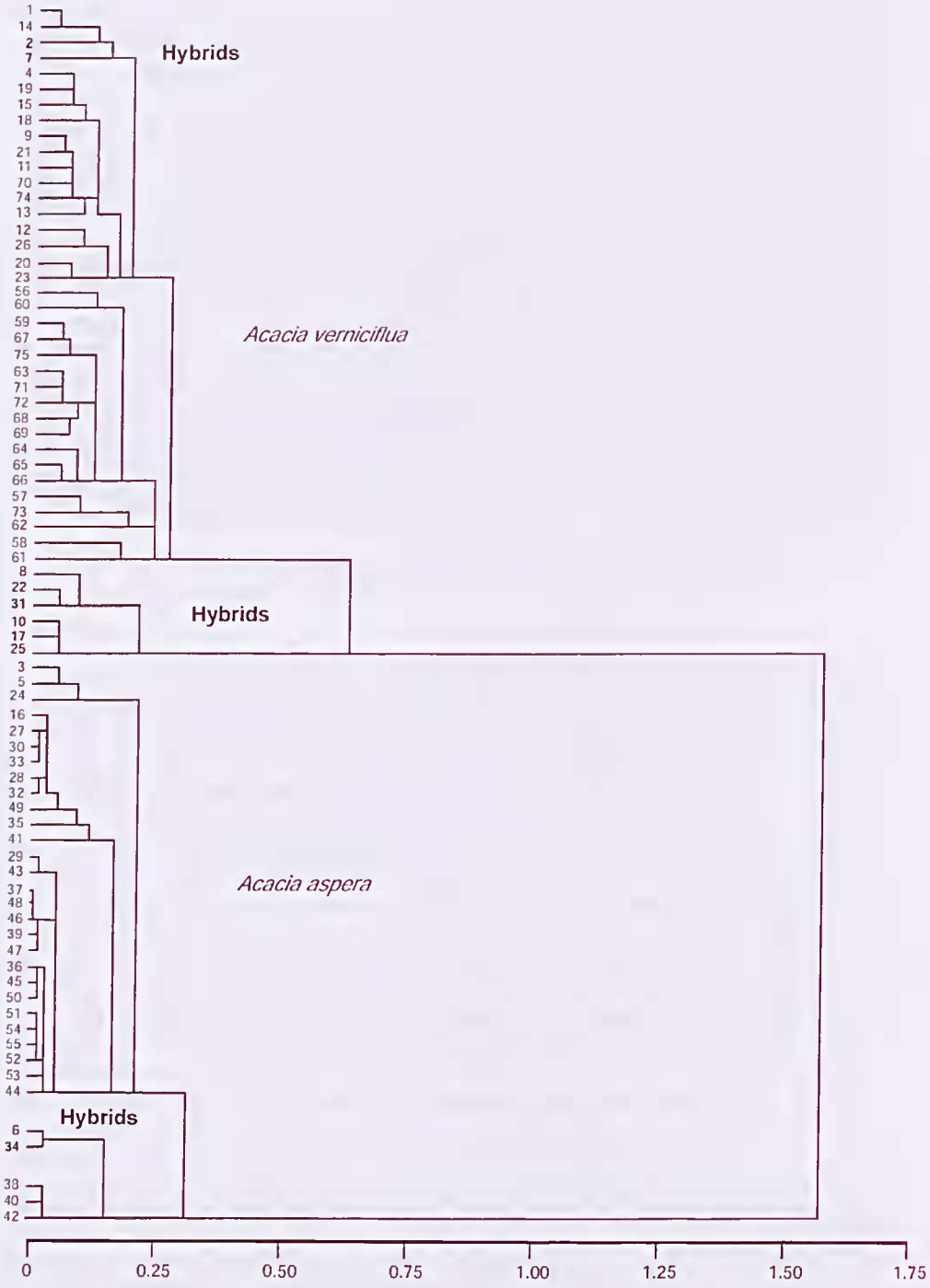

Figure 3. A dendrogram from the agglomerative cluster analysis of 75 specimens (1-35 from the filed site and 36-55 herbarium specimens at MEL). **Suspected hybrids are shown in bold.**

Results

The agglomerative classification (Fig. 3) of all 75 plants (field and herbarium samples) shows three main groups. The groups correspond to *A. verniciflua*, *A. aspera* and a cluster of six intermediate hybrid plants (specimens 8, 10, 17, 22, 25 and 31). This hybrid group clusters at a higher level in the dendrogram with *A. verniciflua* rather than with *A. aspera*. Specimens 2 and 7, categorised in the field as possible hybrids, cluster in the *A. verniciflua* group. Another two specimens thought to be hybrids, 6 and 34, cluster with a subgroup of *A. aspera* (herbarium specimens 38, 40 and 42) that have some phyllodes with two veins in addition to the more typical one-veined phyllodes.

The ordination scatter plot shown in three dimensions (Fig. 4) confirms the three main groups of the dendrogram. All ten suspected hybrids fall in between the two species groups of *A. verniciflua* and *A. aspera*, with specimens 2 and 7 being most like *A. verniciflua* and 6 and 34 being nearer to *A. aspera*. The three herbarium specimens of *A. aspera* from north-east Victoria that had some phyllodes with two veins and some with one vein, form a small cluster next to the main *A. aspera* group.

Phyllode length, width and measure of falcate shape display clearly the intermediacy

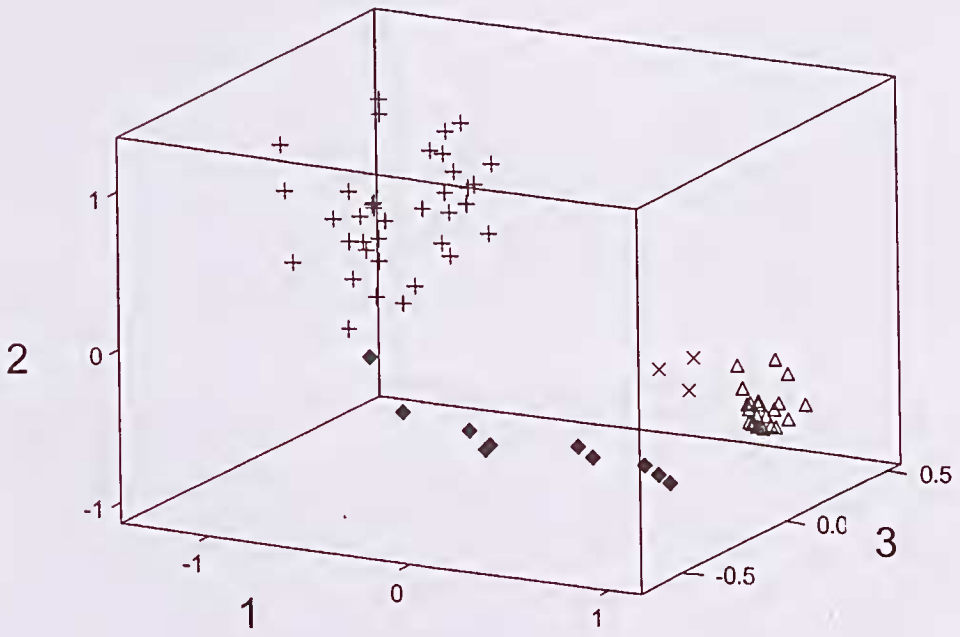

Figure 4. Three dimensional scatter- plot of the 75 specimens. +: *A. verniciflua* group, diamonds: hybrids, triangles: *A. aspera*, x: three herbarium specimens of *A. aspera* from north-east Victoria found to have phyllodes with both 1 and 2 veins.

of the hybrid specimens (Table 2, Fig. 5). The number of main veins for most of the hybrids was variable within the one plant, with some phyllodes having two veins and others only one vein. Whether bracteoles are exserted or not was variable between plants but constant within a plant. The intermediacy of the hybrids is clearly evident in the distribution of unicellular hairs and morphology of glands and glandular trichomes.

Typical of *A. aspera* (Fig. 6a, b) are multicellular glands raised well above the phyllode surface at the tip of a stalk. This contrasts with the presumably homologous but sunken glands of *A. verniciflua* (Fig. 6g), whilst the hybrids had either sessile or shortly stalked glands (Fig. 6c, d, e). The unicellular simple hairs of *A. aspera* are abundant on phyllodes, present but scattered sparsely on the hybrid phyllodes and absent from *A. verniciflua* (Fig. 6a, e, f).

Table 2. Diagnostic characters for parent species and hybrids. Mean values and range (minimum and maximum values) are given.

Character	*A. aspera*	hybrids	*A. verniciflua*
Phyllode length (cm)	1.8 (1.4–2.5)	2.6 (2.3–3.1)	4.8 (2.5–8.5)
Phyllode width (mm)	3 (2–4)	3 (3–3.5)	5 (3–9)
Phyllode curvature (mm)	0 straight	1 intermediate	2–3 subfalcate
Number of main veins	1	1 & or 2	2
Glands stalked or sessile	stalked	sessile or shortly stalked	sessile-sunken
Braeteoles exserted or not	+	+/–	–

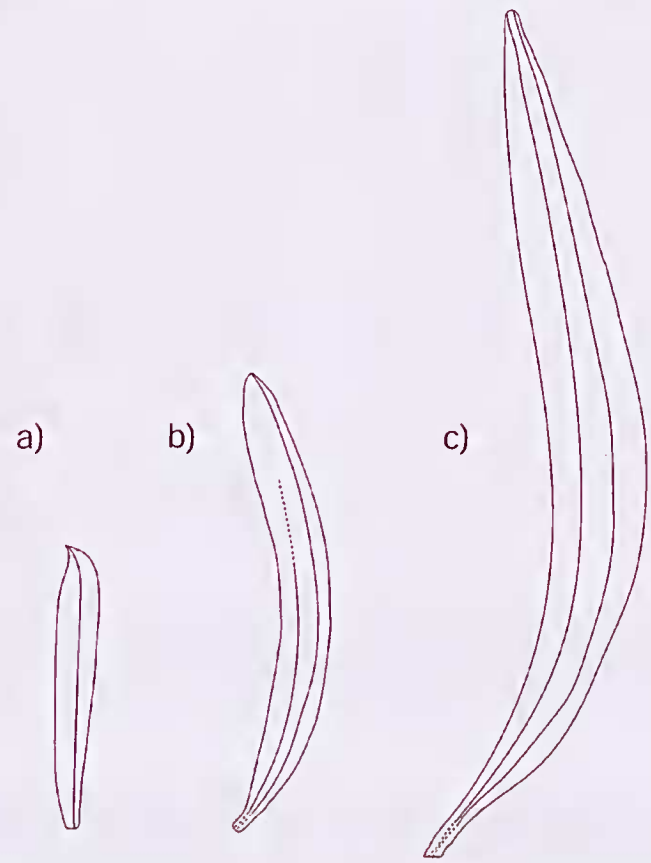

Figure 5. Phyllode shapes of (a) typical *A. aspera* (specimen 35), (b) an intermediate (putative F_1 hybrid, specimen 10) and (c) typical *A. verniciflua* (specimen 14).

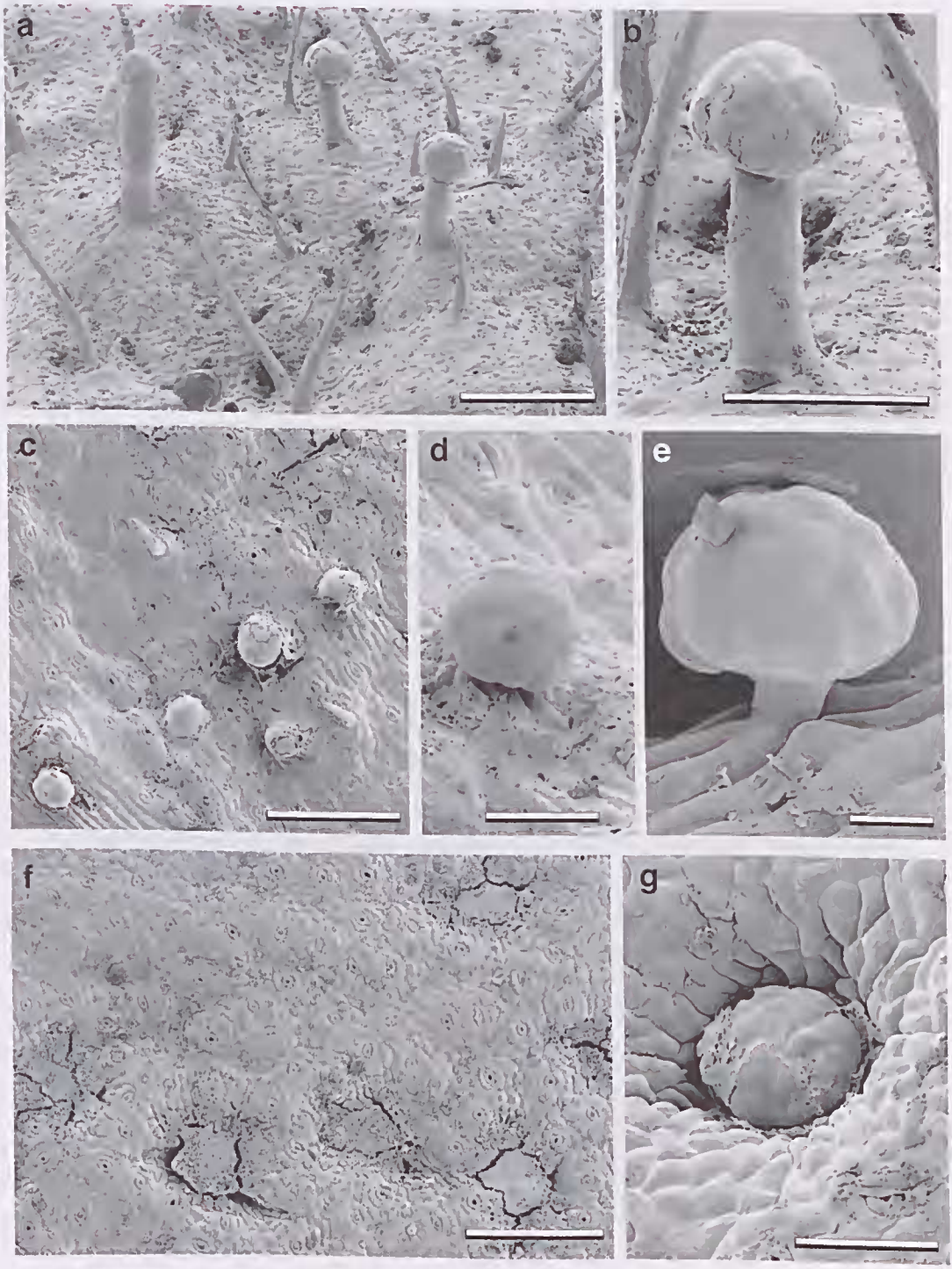

Figure 6. Scanning electron micrographs of the phyllode surfaces of *A. aspera* (specimen 35), a hybrid (specimen 31) and *A. verniciflua* (specimen 23). (a) *A. aspera* stalked resin glands and dense hairs, scale bar = 200mm, (b) at higher magnification, scale bar = 100mm; (c) Hybrid glands and some short hairs of hybrid, scale bar = 200mm, (d) and (e) at higher magnifications, with scale bars =50 and 20mm respectively; (f) *A. verniciflua* with sunken glands covered by resin, which has not dissolved, scale bar = 200mm, (g) sunken gland, scale bar = 50mm.

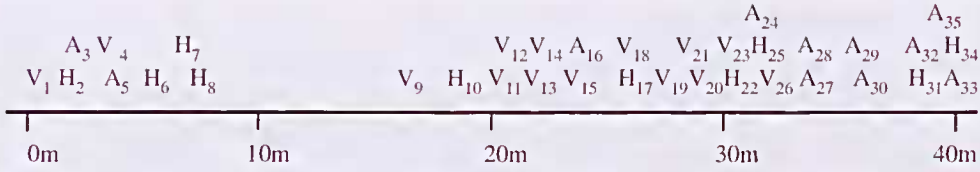

Figure 7. A diagram of the 40m belt transect line showing the relative position of plants sampled in this study; V = *A. verniciflua*, H = hybrids and A = *A. aspera*. Specimen numbers are given beside letters.

Figure 7 illustrates the distribution of the hybrids and the two parent species collected along the transect line at the study site. *Acacia aspera* and *A. verniciflua* are mixed over the study area with hybrids distributed evenly along the 40 metre transect line. For the most part Figure 7 shows that where the hybrids occur there are both *A. aspera* and *A. verniciflua* plants present within a short distance.

Discussion

The morphometric analysis has shown clearly the intermediacy of the suspected hybrid specimens. This supports the hypothesis that six of the field collected specimens are in fact hybrids of *A. aspera* and *A. verniciflua*, probably F_1 progeny. The two suspected hybrids (specimens 2 and 7) that were shown to be more similar to *A. verniciflua* than to the intermediate hybrids may be backcross F_2 progeny. The same explanation is offered for the two suspected hybrids (specimens 6 and 34) that were shown to be more similar to the *A. aspera* group. It is of interest that they were most similar to three herbarium specimens from north-east Victoria that had some phyllodes with two veins, which is suggestive of another instance of *A. aspera* x *A.verniciflua* hybridisation. What controls the development of one or two veins in a single plant requires further examination.

Due to the timing of this study it was not possible to sample flowers, pods or seeds. It is therefore not possible to conclude from this study the fecundity of the hybrids. If the hypothesis that there has been a degree of back crossing is accepted, it would indicate that there is a degree of fertility amongst F_1 progeny.

A link between natural hybridisation and disturbance has long been established (Anderson 1948; Ali & Qaiser 1980; Sedgeley *et al.* 1992; Mayer & Mesler 1993; Hawkins *et al.* 1999). Anderson (1948) put forward the theory of hybridisation of the habitat, which describes the need for an intermediate environment for the survival of the F_1 progeny, and a large amount of variation in the habitat for the survival of a variable F_2 generation. Disturbance of a habitat through the construction of roads may create the micro sites that allow for the establishment of a hybrid swarm (Mayer & Mesler 1993). Such disturbance can create less shading, more water and alter the soil of the area, creating an array of different conditions in which hybrids may grow. The site of the *Acacia* hybrids in the median strip of the Hume Highway is a disturbed site, sunken below the level of the road, acting as a drainage channel. A second site of a possible hybrid swarm further north-east along the Hume highway near Glenrowan has yet to be confirmed.

Conclusion

This study has demonstrated the hybrid status of intermediate plants in a mixed population of *A. verniciflua* and *A. aspera*. This hybrid is a new record for Victoria. Both *A. verniciflua* and *A. aspera* are widely distributed, so the 'genetic purity' of the species is not under great threat. Unless the area is cleared the hybrid population should persist at the site.

Acknowledgments

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Appendix 1

Acacia species	Specimen No.	Collection No.	MEL No.	Location
aspera	36	Beauglehole, A.C. 93423	MEL2022434	10km S.S.E. of Winton
aspera	37	Beauglehole, A.C. 66150	MEL618560	Reef Hill Park
aspera	38	Canning, E.M. 1153	MEL500736	Between Benalla and Wangaratta on Hume Hwy
aspera	39	Hall, N. 79/98	MEL605683	Between Benalla and Wangaratta on Hume Hwy
aspera	40	Hall, N. 79/98	MEL1516433	Hume Highway near Glenrowan
aspera	41	Beauglehole, A.C. 81493	MEL679129	Warby Ranges
aspera	42	Bartolome, M. 193	MEL2155846	16km E.S.E. of Benalla
aspera	43	Fleteher, R.J. 281	MEL2022280	Reef Hill Park
aspera	44	Piesse, A.D.J. 328	MEL1551519	13km N.E. of Benalla on Hume Hwy
aspera	45	Rossiter, N.T. 1	MEL616041	N.E. of Glenrowan
aspera	46	Wallace, G. s.n.	MEL666606	Tarnagulla
aspera	47	Beauglehole, A.C. 69018	MEL618561	4km S.S.E. of Castlemaine
aspera	48	Muir, T.B. 1246	MEL1500071	15 Miles N.E. of Stawell
aspera	49	LeBreton, C. s.n.	MEL234340	Lerderderg Gorge
aspera	50	Clarke, I.C. 2027	MEL1586231	3km W.S.W. of Elphinstone
aspera	51	Walter, C. s.n.	MEL500720	Bendigo
aspera	52	Beauglehole, A.C. 50184	MEL520336	8.5km W.S.W. of Inglewood
aspera	53	Beauglehole, A.C. 50254	MEL520337	27 km N.E. of Stawell
aspera	54	Beauglehole, A.C. 38930	MEL500735	Lightning Hill Reservoir, Bendigo
aspera	55	Beauglehole, A.C.18928	MEL500731	Llanelly
verniciflua	56	Court, A.B. & Willis, J.H.	MEL1529029	5 miles E. of Wilby
verniciflua	57	Fleteher, R.J. 263	MEL2021041	30km S.E. from Wangaratta
verniciflua	58	Piesse, A.D.J. 426	MEL1555746	10.4km S. of Whitfield
verniciflua	59	Willis, J.H. s.n.	MEL502697	Central Summit of Mt. Ida, near Heatheote
verniciflua	60	Crawford, I. 1816	MEL712592	Dookie Agriculture College
verniciflua	61	Fleteher, R.J. 287	MEL2022274	Chiltern State Park, near Cyanide Dam
verniciflua	62	Fletcher, R.J. 288	MEL2022273	Chiltern State Park, near Cyanide Dam
verniciflua	63	Beauglehole, A.C. 80343	MEL679039	Boweya Flora & Fauna Reserve
verniciflua	64	Fletcher, R.J. 289	MEL2022272	Chiltern State Park, near Cyanide Dam
verniciflua	65	Beauglehole, A.C. 92378	MEL2022424	Snowfields, Mt Buffalo Reference Area
verniciflua	66	Beauglehole, A.C. 16962	MEL1516197	Mt. Victory, Grampians
verniciflua	67	Beauglehole, A.C. 16964	MEL563430	Mt. Victory, Grampians
verniciflua	68	Parfett, S.T.W. 56	MEL1537955	200m S.S.E. of Scout Camp, Grampians
verniciflua	69	Beauglehole, A.C. 17456	MEL563429	Grampians
verniciflua	70	Unknown	MEL1529069	Cultivated
verniciflua	71	Hicks, A.J. s.n.	MEL1529027	Rodes Gap, Grampians
verniciflua	72	Muir, T.B. 2146	MEL1529049	32 miles S. of Horsham
verniciflua	73	Court, A.B. s.n.	MEL1516049	Near Chiltern
verniciflua	74	Walter, C. s.n.	MEL1528783	Dandenong Ranges
verniciflua	75	Walter, C. s.n.	MEL1528778	Dandenong Ranges